bio-protocol Preprint

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Bacterial isolation, genome amplification and DNA extraction

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An abbreviated version of this protocol was published in eLIFE in Nov 2018

Reconstructing the functions of endosymbiotic Mollicutes in fungus-growing ants

DOI: 10.7554/eLife.39209

Detailed protocol

- Isolate ant host tissue or collect fecal droplets
- Suspend isolated material in 1000 µL cold SPG Buffer (218 mM sucrose, 3.8 mM KH_2PO_4, 7.2 mM K_2HPO_4, 4.9 mM l-glutamate, pH 7.2) or homogenize it (in cold SPG buffer) using an ice cold Wheaton glass homogenizer
- Centrifuge twice at 4 ˚C for 15 min at 3,200 g
- Pass the supernatant through a 5 µm (Acrodisc) syringe filter first, then a 2.7 µm (Whatman) syringe filter, and finally through a 1.3 µm (Acrodisc) syringe filter.
- Centrifuge at 4 ˚C for 20 min at 18,000 g
- Resuspend the pellet (bacterial cells) in 5 µl SPG buffer
- Use 1 µl for Multiple Displacement Amplification (MDA) to obtain whole genomic DNA using the Qiagen REPLI-g Midi Kit following the manufacturer's instructions

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sapountzis, P. and Sapountzis, P. (2021). Bacterial isolation, genome amplification and DNA extraction. Bio-protocol Preprint. bio-protocol.org/prep948.
2. Sapountzis, P., Zhukova, M., Shik, J. Z., Schiott, M. and Boomsma, J. J.(2018). Reconstructing the functions of endosymbiotic Mollicutes in fungus-growing ants. eLIFE. DOI: 10.7554/eLife.39209